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Attention : Vittiorio Sgaramella 12/17 92
Dept. of Genetics and Microbiology
University of Pavia

From: Greg Tombline
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Dear Vittorio,

Hope that all is well with you, I have recovered completely and back into the swing of things.... and as you may notice, am becoming computer literate. The experiments are going OK ... I sequenced the mutants with sequenase and got a clear, readable pattern - the mutants were detectable, and on the same gel attempted the fingerprinting (both sequencing and the fingerpr. done with both P3 and P4). The F.P. was not very visible- I acted foolishly and did not extract and precipitate. Consequently, I ran another set of reactions and will extract and precipitate today, and possibly load another gel tonight or tomorrow morning. The last gel consisted of Hydrolink with regular spacers (ie. not wedged) and freshly obtained TEMED - so the resolution was good (publishable) but could be better if I run it longer. Fortunately, I still have quite a bit of the sequencing to reload. We'll have the results by monday if all goes well with the F.P. As for the nicking/ endonuclease experiments ... I was able to kinase the primers 1, 2, 3, and 4 and use them in a PCR which was succesful (photocopy of autorad. enclosed). Ihave yet to purify these PCR's but plan on this today since I just did the control of the alpha labeled PCR of 1-2 , 3-4 last night , so I can purify them with Quiagen spin columns in parallel. I also wanted to tell you that I ran the kinased primers on a 10% Hydrolink gel (normal spacers) and am glad to say that there does not appear to be an enormous amount of higher mol. wt. material conaminating them . This was exposed to the phosphorimager-- this allowed me to integrate the # of counts over the area of each band and at least get a feel of thepurity of each primer relative to one another.

As for David's arena... the grant proposal is out, and I've been working on controls.... We've shown that RNA does not seem to be mutagenic in trans under conditions where the RNA was added (after synthesis and extraction/ ppt) to DNA primed synthesis as well as when no primer was added and synthesis allowed to occur. There is also no effect of annealing of the RNA without synthesis- the

progeny are completely restricted. As for RNA primed PCR ... although it worked seemingly well three times with different RNA's - in which alpha 35S labeled RNA was seen by autorad. in the product, the effect was not seen using the most recent RNA, so.... I plan on using some of the old RNA to see if it is repeatable, as well as make more RNA... but for now, there are more pertinent experiments. The final ? control for the RNA as a mutagen stuff is underway as I write.... Anealing and subsequent synthesis were done with RNA's and DNA primers as usual, but in parallel, the same set were previously exposed to alkali. These will be electroporated, and hopefully the effect of the RNA will disappear while the DNA mutant primer will still exert the same mutagenic effect.. This will show that the DNase treatment that follows transcription does not leave DNA which could act as a primer and even transfer the specific mismatched information.... I'll let you know how it goes.

Mike told me to mention that the task you inquired about would only take a day or two.... One more thing, please send an E-mail address.. Oh, and Joyce found the book - thanx. So let me know what's happening, and stay well. Give my best to Puala and the girls....

Greg
Grosso Bidone